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Prevalence of exposure to bovine viral diarrhoea virus (BVDV) and bovine herpesvirus-1 (BoHV-1) in Irish dairy herds

R.G. Sayers [a,*], N. Byrne [a], E. O'Doherty [a], S. Arkins [b]

[a] Animal & Bioscience Research Department, Animal & Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland
[b] Department of Life Sciences, University of Limerick, Limerick, Ireland

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ABSTRACT

Bovine viral diarrhoea virus (BVDV) and bovine herpesvirus 1 (BoHV-1) are contagious bovine viral agents. The objectives of this study were to use quarterly bulk milk and 'spot' testing of unvaccinated youngstock to establish the national prevalence of exposure to BVDV and/or BoHV-1 in Irish dairy herds. Seasonality of bulk milk ELISA results was also examined. From a geographically representative population of 305 dairy herds, 88% and 80% of herds yielded mean annual positive bulk milk readings for BVDV and BoHV-1, respectively. Of these, 61% were vaccinated against BVDV and 12% against BoHV-1. A total of 2171 serum samples from weanlings having a mean age of 291 days yielded 543 (25%) seropositive for BVDV, and 117 (5.4%) seropositive for BoHV-1. A significant seasonal trend in bulk milk antibody ELISA readings and herd status was recorded for BVDV, with more herds categorised as positive in the latter half of the year.

1. Introduction

Bovine viral diarrhoea (BVD), caused by BVD virus (BVDV), and infectious bovine rhinotracheitis (IBR), caused by bovine herpesvirus 1 (BoHV-1), are highly contagious viral diseases of cattle (Moennig et al., 2005; Muylkens et al., 2007; Nandi et al., 2009). Both exhibit a worldwide distribution (Lindberg et al., 2006; Thiry et al., 2006) and are listed as notifiable diseases by the Office International des Epizooitic[1] (OIE). Although OIE-listed diseases, compulsory national control programmes for BVDV and BoHV-1 do not exist in many countries (Ackermann and Engels, 2006; Heffernan et al., 2009).

Where regulation does exist, successful BVDV eradication has been achieved through the use of 'test and cull' protocols involving removal of persistently infected (PI) individuals (Heffernan et al., 2009; Lindberg et al., 2006; Moennig et al., 2005; Presi et al., 2011; Ridpath, 2012; Ståhl and Alenius, 2012; Valle et al., 2005). In the case of BoHV-1, vaccination with marker/DIVA (**D**ifferentiating **I**nfected from **VA**ccinated) vaccines (Mars et al., 2001; Nandi et al., 2009; Nardelli et al., 2008; van Oirschot, 1999) constitutes the primary method of control and eradication in high prevalence regions. In January 2013, a mandatory national eradication programme for BVD, coordinated by the Animal Health Ireland (AHI), was introduced in the Republic of Ireland (Graham et al., 2013). As yet, a co-ordinated approach to BoHV-1 control does not exist in Ireland.

In order to determine the necessity for, and measure ongoing success of an eradication programme, it is useful to conduct prevalence studies to obtain baseline data (Heffernan et al., 2009; Lindberg et al., 2006; Lindberg and Alenius, 1999; Paisley et al., 2001). National prevalence studies, however, are often prohibitively expensive (Thrushfield, 2005). The advent of bulk milk testing overcomes this issue and reliable antibody detection bulk milk test procedures have been developed for both BVDV and BoHV-1 (Beaudeau et al., 2001; Nylin et al., 2000). Bulk milk analysis for BVDV antibodies, however, does not readily distinguish between vaccinated and unvaccinated herds (Lindberg et al., 2006). This issue has been overcome in the case of BoHV-1 with the advent of BoHV-1 gE-deleted DIVA vaccines. Due to legislative requirements,[2] all BoHV-1 vaccines administered in the Republic of Ireland since December 31, 2004 are DIVA vaccines (Simon, 2004).

Additionally, bulk milk BVD antibody readings may reflect historical rather than current herd viral status (Brülisauer et al., 2010; Lindberg and Alenius, 1999). To overcome this issue, it is useful to test unvaccinated homeborn youngstock (weanlings) for antibodies against BVDV, i.e. a 'spot test' (Houe, 1992, 1994; Mars and Van Maanen, 2005). Positive antibody readings in this population, once maternal antibodies have dissipated, can be indicative of current

* Corresponding author. Animal & Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland. Tel.: +353(0)2542215; fax: +353(0)2542385.
E-mail address: riona.sayers@teagasc.ie (R.G. Sayers).
[1] www.oie.int.
[2] Diseases of Animals Act 1966; Control on Animal and Poultry Vaccines Order 2002; S.I. 528 of 2002 www.irishstatutebook.ie.

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or recent viral circulation (Houe, 1992, 1994; Lindberg and Alenius, 1999), and as such provide a useful adjunct to bulk milk testing.

Although preliminary surveillance studies have indicated high levels of both BVDV and BoHV-1 in the Irish national cattle population (Cowley et al., 2011, 2012; O'Grady et al., 2008; O'Neill et al., 2009), national prevalence data for BVD and BoHV-1 exposure among a geographically representative sample of Irish dairy farms are not available. In addition, evaluation of longitudinal BVD and BoHV-1 bulk milk data over a single lactation in a predominantly spring-calving dairy system has not been reported previously. The primary objective of this study, therefore, was to use bulk milk analysis and spot testing of Irish dairy herds to generate national prevalence data for both BVD and BoHV-1, while investigating the usefulness of this diagnostic strategy in an Irish context.

2. Materials and methods

2.1. Sample population and survey

The study was licenced by the Irish Department of Health and Children in 2009, meeting all legislative requirements for research involving animals in the Republic of Ireland at the time of the study.

A detailed description of the sample population used in this study is outlined in O'Doherty et al. (2013). Briefly, stratified proportional sampling based on herd size and geographical location was used to randomly select and invite 500 herds from the Irish Cattle Breeding Federations (ICBF) database to partake in the study on a non-incentivised basis. Over the 2009 lactation, four bulk milk samples (23 March, 8 June, 31 August and 2 November) were submitted by post in a standardised kit from each participating farm. Each study farm was visited between October 2009 and January 2010 to collect blood samples by coccygeal venepuncture from 20% of the replacement heifer group (weanlings for spot test) on each farm, with a minimum of five weanling heifers sampled on each farm. All heifers were homeborn and not vaccinated against BVDV. Where possible, only weanlings over 270 days of age were sampled, although not achievable in all cases. Accurate weanling age based on calf registration data was downloaded from the ICBF database.

2.2. Sample analysis

Commercially available enzyme linked immunosorbent assay (ELISA) kits were used to test bulk milk samples for the presence antibodies against: (i) BVD p80 (NS3) protein, (Institut Pourquier, France); (ii) Ultrapurified IBR lysate (Institut Pourquier, France) in BoHV-1 unvaccinated herds; and (iii) IBRgE, (IDEXX laboratories, USA) in BoHV-1 vaccinated herds. Weanling serum samples were also tested for antibodies against BVD p80, ultrapurified IBR lysate, and IBRgE with serum adapted positive cut-off values applied as outlined by kit manufacturers (Table 1). All analyses were completed by commercial accredited laboratories; BVD p80 and IBR lysate by National Milk Laboratories Ltd. (UK), and IBR gE by Enfer Diagnostics Ltd. (Ireland).

2.3. Herd classification

Calving data from the ICBF were used to determine calving-season of each herd (spring-calving and non-spring-calving, i.e. spring-autumn [SA] and year-round [YR]) as described by O'Doherty et al. (2013). Vaccination status (vaccinated [V] and unvaccinated [UV]) was determined by questionnaire, with date of vaccination, product used, and class of animal vaccinated (cows, yearling-heifers, weanlings) recorded. In all cases, kit-manufacturer positive cut-off values were applied to ELISA outputs in order to classify herds as 'positive' or 'negative'. Herds were classified as positive or negative at each of the four sampling time points (longitudinal data). Additionally, a mean annual ELISA result for each herd (herd status data) was calculated to provide an overall bulk milk classification for each herd. Herds were also categorised on the basis of combined BVDV and BoHV-1 bulk milk antibody status, i.e. negative for both viral antibodies, positive for BoHV-1 and negative for BVD, negative for BoHV-1 and positive for BVD, and positive for both viral antibodies.

Finally, herds were classified with regard to the presence of seropositive unvaccinated weanlings. Two datasets were constructed with weanlings either categorised 'positive aged ≥180 days of age' or 'positive aged ≥270 days of age' to both assess and minimise potential interference from maternally derived antibodies (MDAs) (Fulton et al., 2004). Herds having at least one weanling serologically positive for either BVDV or BoHV-1 were classified as having 'evidence of recent viral circulation' (RVC) (Houe, 1992; Handel et al., 2011). Herds not recording a positive weanling or recording a positive weanling under either 180 or 270 days of age, depending on the dataset, were classified as 'not having evidence of recent viral circulation' (NRVC).

2.4. Data analysis

Descriptive analysis and graphical representations were completed in Excel (MS Office 2010). Normality of the data was assessed visually using ladder of powers histograms, with normality of residuals assessed using normal probability plots and kernel density estimate plots constructed in Stata (Version 12). True prevalence was calculated using the Rogan–Gladen estimator in the survey toolbox version 1.04 (www.ausvet.com.au (Cameron, 1999)). Pearson's chi-squared, Fisher's exact, univariable and multivariable logistic regression, generalised estimating equations (GEE), multinomial logistic regression, Wilcoxon rank sum, and Hosmer–Lemeshow test of goodness of fit analyses were carried out using Stata (Version 12).

Seasonal trends in true prevalence for both diseases were tabulated. In addition, box plots of %inhibition, %S/P, and S/N ratio for BVDp80, IBR lysate, and IBR gE, respectively, at each sampling time

Table 1
ELISA kit performance data and positive cut-off values for BVD and BoHV-1assays used in this study.

Test	BVD P80 Milk	IBR Lysate Milk	IBR gE Milk	BVD P80 Serum	IBR lysate Serum	IBR gE Serum
Sensitivity	95.0%	100%	72.0–88.4%	97.6%	98.7%	100%
Specificity	97.7%	99.6%	100%	97.3%	99.9%	>99%
Positive cut-off	≥55	≥25	≤0.8	>60	>55	≤0.60
(Kit)	%Inhibition[a]	%S/P[b]	S/N ratio[c]	%Inhibition[a]	% S/P[b]	S/N ratio[c]
Within-herd prevalence	≥30%[d]	Not available	10.0–15.0%[e]	n/a	n/a	n/a

[a] %Inhibition = [1 – (OD 450 of analysed sample / mean OD 450 of negative control)] × 100.
[b] %S/P = (OD 450 of sample – OD 450 of negative control) / (mean OD 450 of positive control – OD 450 of negative control) × 100.
[c] S/N ratio = (sample mean – absorbance 650 nm)/negative control mean.
[d] Beaudeau et al., 2001.
[e] Wellenberg et al, 1998; Kramps et al., 1994.

point were constructed. Two BVD datasets were examined by GEE and logistic regression, i.e. all study herds regardless of BVD vaccination status, and BVD unvaccinated herds only.

Longitudinal data were used for the purposes of GEE analysis. To examine seasonal effects on bulk milk analysis, a univariable analysis of bulk milk results (constructed as both categorical [positive *vs*. negative] and continuous [ELISA readings] variables) and sampling time point was completed (Woodbine et al., 2009).

Examination of additional influences on bulk milk longitudinal data (both categorical and continuous) by a number of independent variables was also completed. Independent variables examined included region (high density dairy *vs*. low density dairy), herd size (31–65 cows *vs*. 66–99 cows *vs*. > 99 cows), calving season (spring-calving *vs*. non-spring-calving), type of farming enterprise (dairy livestock only *vs*. mixed livestock), vaccination status (V *vs*. UV), and recent viral circulation (RVC ≥180 days or RVC ≥270 days *vs*. NRVC ≥180 days or NRVC ≥270 days). A total of four datasets were analysed by GEE to account for BVD vaccination status (V, UV) and differing weanling age groups (≥180 and ≥270 days of age).

Logistic regression was used to examine associations between recent viral circulation status (RVC ≥180 or ≥270 days *vs*. NRVC ≥180 or ≥270 days) and vaccination status (V *vs*. UV), the dependent variables, and region, calving-season, enterprise-type, herd-size, and annual mean bulk milk herd status (independent variables). Four datasets were constructed to account for BVD vaccination status (V, UV) and positive-weanling age (≥180 or ≥270 days).

Multinomial logistic regression analysis was completed on combined BVDV and BoHV-1 bulk milk status with region, herd-size, enterprise-type, calving-season and recent viral circulation status as independent variables.

For all GEE analyses, herd was included as a repeated measure and an exchangeable correlation used. A binomial distribution was assumed and a logit link function applied for categorical data; a Gaussian distribution and identity link function were used for continuous data. All regression models were constructed by first completing a univariable analysis. Those variables recording p values of ≤0.15 in univariable analyses were included in multivariable models. A manual backwards elimination with a forward step was used to build models with variables recording p values of ≤0.05 maintained. Second level interactions deemed biologically significant were also included. The overall fit of regression models was assessed using the Hosmer–Lemeshow good of fit test following ordinary logistic regression (categorical variables). Normality of residuals was assessed following logistic regression (categorical variables) and linear regression (continuous variables).

To examine differences in bulk milk readings between RVC herds and NRVC herds, a Wilcoxon rank sum analysis was completed for each sampling time point. Herds were examined based on vaccination status (V and UV) for both BVDV and BoHV-1, and a third analysis was completed for BVDV where all herds regardless of vaccination status were included. Both viral circulation infection classifications were examined, i.e. herds with positive weanlings greater than 180 or 270 days of age.

3. Results

A total of 312 herds were recruited to the study (Fig. 1), yielding a sufficient sample size to achieve a 95% confidence level and precision of 5% for a national dairy herd population of approximately 18,000 herds with an expected national prevalence of 70%. A complete set of four bulk milk samples was not achieved for four farms and vaccination data were not returned by three farmers. Of the herds recruited to the study, 305 herds were therefore suitable for final analysis. Weanling ages were unavailable for eleven herds and these data were excluded from statistical analysis.

Study herds have previously been shown to geographically represent the Irish national dairy farm population (O'Doherty et al., 2013). The distribution of study herds across region, herd size, calving season, and type of enterprise is included in Table 2. Approximately 60% of study participants were vaccinated against BVDV using inactivated vaccines, with 12.5% vaccinating against BoHV-1 using DIVA vaccines. A total of 33 study farms administered vaccines for both BVDV and BoHV-1.

3.1. Prevalence of bulk milk positive herds

The apparent prevalence (Ap) of bulk milk antibody positive herds for BVDV and BoHV-1 was approximately 88% (80% in unvaccinated herds) and 80% (78% in unvaccinated herds), respectively. True prevalence (Tp) and 95% CI at each sampling time point and across vaccination status is outlined in Table 3. Concurrent exposure to BVDV and BoHV-1 was detected in 72% of herds, with only 10 herds recording bulk milk seronegative status for BVDV and BoHA-1. Seasonal trends in ELISA readings for each disease are included in Fig. 2.

3.2. Seasonal pattern of bulk milk results

Univariable GEE analysis highlighted significant seasonal differences in BVDV and BoHV-1 herd status examined as both categorical and continuous variables (Supplementary Table S1).

Multivariable analysis of exposure to BVDV and BoHV-1 as continuous variables highlighted a general increase in ELISA readings as the year progressed for both BVD and BoHV-1 (Table 4). When examined as categorical variables, a significant seasonal effect was only observed for BVD bulk milk herd status and a significant interaction between enterprise type and sampling time point was highlighted (Table 4). Herds with a mixed livestock enterprise, in general, were more likely to record a BVD positive bulk milk result in the latter half of the year. This association was apparent regardless of BVD vaccination status. Visual examination of normal probability plots and kernel density plots of residuals did not highlight evidence of non-normality. Goodness of fit analyses for

Table 2
Distribution of study herds across region, herd size, calving-season and enterprise-type.

Region[a] (density)	Counties represented	Herd size (cows)			Calving season		Enterprise[c]	
		31–65	66–99	>99	Spring	SA/YR[b]	Dairy	Mixed
Region 1 (Low) 32.5%	Carlow, Cavan, Clare, Donegal, Dublin, Galway, Kildare, Laois, Leitrim, Longford, Louth, Mayo, Meath, Monaghan, Offaly, Roscommon, Sligo, Westmeath, Wexford, Wicklow	n = 29 9.5%	n = 26 8.5%	n = 44 14.4%	n = 75 24.6%	n = 24 7.9%	n = 52 17%	n = 46 15.1%
Region 2 (High) 67.5%	Cork, Kerry, Kilkenny, Limerick, Tipperary, Waterford, Limerick	n = 52 17.0%	n = 72 23.6%	n = 82 26.9%	n = 190 62.3%	n = 16 5.2%	n = 88 28.9%	n = 118 38.7%

[a] Regions were chosen to correspond with Irish dairy farm distribution (Sayers et al., 2013) and to represent a natural geographical spread.
[b] SA/YR represents Spring-Autumn and Year-Round calving seasons.
[c] Type of enterprise was not supplied by a single participant.

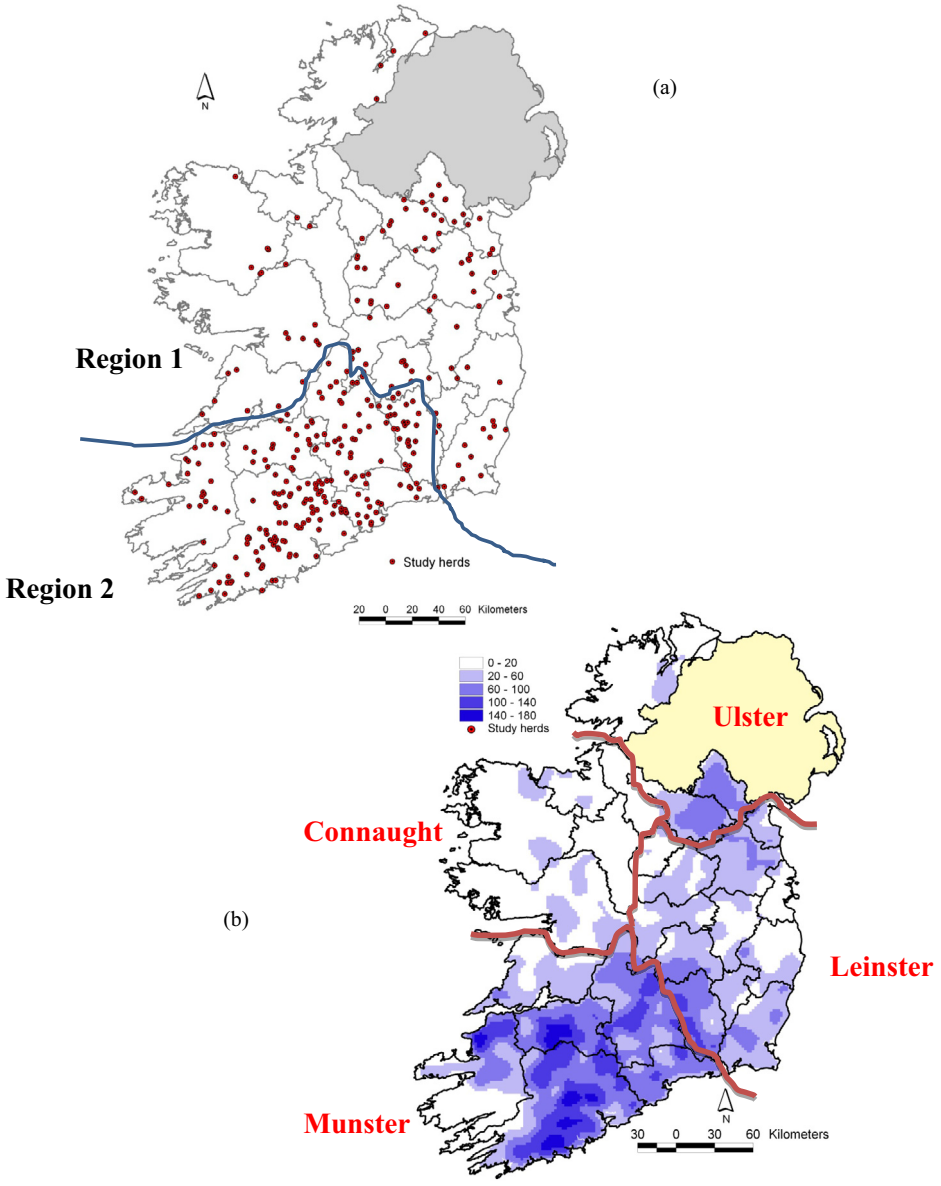

Fig. 1. (a) Location of study herds and, (b) representation of the density of animals on dairy farms in Ireland.

categorical data using ordinary logistic regression yielded non-significant values ranging from p = 0.286 to p = 0.878.

Examination of individual herd results highlighted 15 herds which recorded elevated BVD readings in August and November samplings (Table 5), four of which (herds 67, 263, 273, 275) may be suggestive of introduction of BVD virus to the lactating herd. Herd 263 reported diarrhoea, fever, and milk drop across the lactating herd over the month of July prior to submission of the August bulk milk sample. An additional herd (herd 142) administered vaccine in September which may account for the elevated reading in November. Remaining herds, although having progressed to positive herd status in either August or November, did not record sufficiently elevated readings to be regarded as biologically significant given a positive cut-off of 55% inhibition.

3.3. Youngstock serological status

A total of 2171 serum samples from weanlings having a mean age of 291 days (range 109 to 549) were analysed, with 543 testing seropositive for BVDV and 117 testing seropositive for BoHV-1. The

Table 3
True prevalence (Tp) and 95% confidence interval (CI) of exposure to BVDV and BoHV-1 in Irish dairy herds of varying vaccination status at each sampling time point.

Sample date	BVD		IBR	
	Tp	95% CI (%)	Tp	95% CI (%)
All herds	n = 305			
March	90.5	86.4,94.7		
June	90.5	86.4,94.7		
August	96.2	92.8,99.6		
November	94.4	90.8,98.1		
Unvaccinated herds only	n = 113		n = 269	
March	81.5	73.3,89.8	80.2	75.8,84.7
June	83.4	75.4,91.4	79.6	75.1,84.1
August	94.9	89.0,100.0	77.3	72.6,82.0
November	93.0	86.6,99.3	79.6	75.1,84.1
*gE herds**			n = 36	
March			100	n/a
June			100	n/a
August			100	n/a
November			100	n/a

* Describes herds vaccinated with a BoHV-1 DIVA vaccine and tested using a gE ELISA.

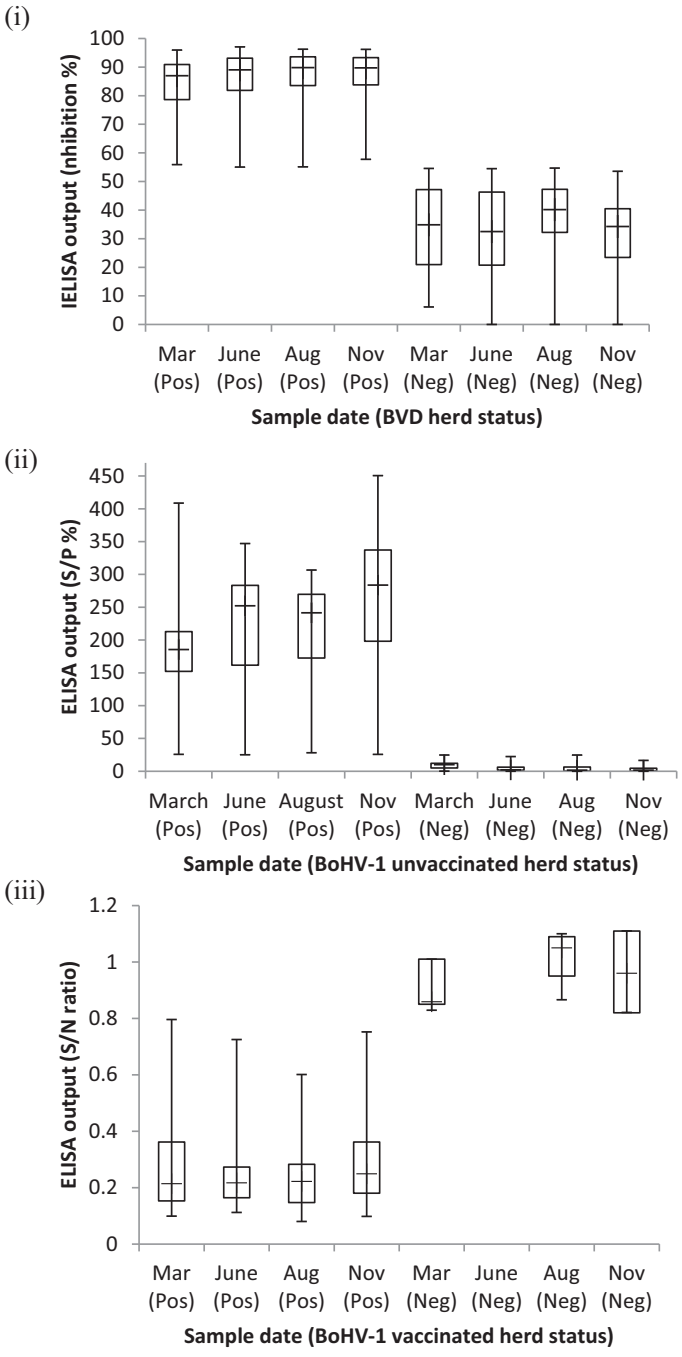

Fig. 2. Box plots outlining seasonal trends[a] in bulk milk ELISA readings across positive (Pos) and negative (Neg) (i) BVD, (ii) BoHV-1 unvaccinated, and (iii) BoHV-1 vaccinated herds in 2009.
[a]Mar: March; June: June; Aug: August; Nov: November.

age profile and BVDV/BoHV-1 serological test status of study weanlings is included in Fig. 3. At least one seropositive weanling over 180 days of age was identified in 119/294 study herds in the case of BVDV, and 24/294 in the case of BoHV-1. If an age limit of 270 days was applied, 96/294 herds recorded a single seropositive BVDV weanling and 18/294 a BoHV-1 seropositive weanling (Fig. 4). A total of 10 herds recorded weanlings ≥180 days old seropositive for both BVDV and BoHV-1, and 8 herds having concurrently seropositive weanlings if an age limit of ≥270 days was applied.

3.4. Associations between herd demographics and bulk milk status

Regional differences in both BVDV and BoHV-1 herd classification were highlighted by multivariable GEE analysis (Table 4), though this was not consistent across all models. Study herds in the most dairy dense region of Ireland (Region-2) were almost twice as likely to be categorised as BVDV antibody positive over those in Region-1 when all herds, regardless of vaccination status, were included in the model. The reverse was highlighted for BoHV-1, where herds in Region-1 (the least dairy dense part of Ireland) were found to be almost twice as likely as those in Region-2 to be categorised as positive. Herd size was significantly associated with BoHV-1 herd status, with larger herds (>99 cows) approximately four times more likely than smaller herds to be categorised positive. Finally, vaccination was associated with positive herd status for both BVD (OR = 4.29) and BoHV-1 (OR = 31.88), with vaccinating herds more likely to be categorised positive.

3.5. Associations between recent viral circulation status, vaccination, and herd demographics

All models examined highlighted a significant association between BVDV bulk milk antibody status and BVDV RVC, herds having evidence of recent BVDV circulation at least three times more likely to be bulk milk positive than those herds recording no seropositive weanlings (Supplementary Table S2 and Table 6). No such association was highlighted in the case of BoHV-1 bulk milk antibody positive herds. A tendency for larger herds to have RVC for either BVDV or BoHV-1 was highlighted, with non-spring-calving herds also more likely to contain BVDV seropositive weanlings (Table 6).

Larger herds were more likely to vaccinate for both BVDV and BoHV-1 in this study population. In addition, herds vaccinating for BVDV were more likely to also vaccinate for BoHV-1 and vice versa (Table 7). There were tendencies for herds that were BoHV-1 bulk milk antibody positive to vaccinate for BVDV and for non-spring-calving herds to vaccinate for BoHV-1.

3.6. Multinomial logistic regression analysis

Multinomial logistic regression highlighted that compared to herds bulk milk antibody negative for both BVDV and BoHV-1, larger herds were more likely to be antibody positive for BoHV-1 and negative for BVDV (OR = 3.70, 95% CI = 1.52, 9.04, P = 0.004) and antibody positive for both BVDV and BoHV-1 (OR = 2.67, 95% CI = 1.23, 5.81, P = 0.013). In addition, compared to antibody negative herds, those operating mixed livestock enterprises tended to be over three times more likely than dairy-only herds to present with exposure to one (OR = 4.04, P = 0.071, BoHV-1; OR = 3.35, P = 0.10, BVDV) or both viral pathogens (OR = 4.84, P = 0.024).

3.7. Wilcoxon rank sum analysis

A significant difference was highlighted between RVC and NRVC herds in terms of BVDV bulk milk %inhibition readings when all herds were included in the analysis regardless of vaccination status, with *z* values ranging from –2.718 to –3.864 (Supplementary Table S3). A similar result was generated for BVDV unvaccinated herds alone, with *z* values ranging from –3.901 to –4.617. No significant difference in %inhibition was highlighted between BVDV vaccinated RVC vs. NRVC herds, however. An analysis of BoHV-1 yielded similar results, with a significant difference in ELISA outputs highlighted for unvaccinated herds (with the sole exception of the March sample), but no significant difference in ELISA readings between vaccinated RVC and NRVC herds (Supplementary Table S3).

Table 4
Multivariable GEE analysis of BVDV and BoHV-1 herd classification.

Dependent variable *Categorical*	Independent variable	Odds ratio	Confidence interval (95%)	p value	Model (p value)
BVD status	Region 2 vs. Region 1	2.02	1.09,3.84	0.027	Region
POSITIVE vs. NEGATIVE	Vaccinated vs. Unvaccinated	4.29	1.09,8.06	<0.001	BVD vaccination
(All herds included regardless of	Mixed August vs. Mixed March	3.19	1.54,6.58	0.002	Sampling time point
vaccination status)	Mixed November vs. Mixed March	2.04	1.08,3.83	0.027	Enterprise
	Mixed August vs. Mixed June	1.96	0.96,4.03	0.064*	Sampling time point*enterprise (P < 0.001)
BVD classification	Mixed August vs. Mixed March	3.46	1.45,8.25	0.005	Sampling time point
POSITIVE vs. NEGATIVE	Mixed November vs. Mixed March	2.38	1.07,5.29	0.032	(P < 0.001)
(UV herds only)					
IBR classification	Region 1 vs. region 2	1.77	0.98,3.18	0.056*	Region
POSITIVE vs. NEGATIVE	Vaccinated vs. Unvaccinated	31.88	0.92,1102.57	0.057*	IBR vaccination
(All herds included)	>99cows vs. 31–65 cows	3.66	1.82,7.37	<0.001	Herd size
	>99cows vs. 66–99cows	4.15	2.11,8.19	<0.001	(P < 0.001)
Continuous		**Co**[a]	**CI**	**p value**	**Model**
BVD ELISA readings	Region 2 vs. Region 1	4.58	0.44,8.72	0.030	Region
	Vaccinated vs. Unvaccinated	10.72	6.76,16.67	<0.001	BVD vaccination
	August vs. March	4.53	2.66,6.39	<0.001	Enterprise
	November vs. March	2.73	0.86,4.59	0.004	Sampling time point
	Mixed June vs. Mixed March	4.26	1.71,6.81	0.001	Enterprise*sampling time point
	Mixed August vs. Mixed March	3.04	0.49,5.59	0.019	P < 0.001
	Mixed November vs. Mixed March	4.30	1.75,6.84	0.001	
BoHV-1 ELISA readings	June vs. March	22.29	14.31,30.29	<0.001	Sampling time point
(excluding vaccinated herds i.e. those	August vs. March	14.29	6.29,22.27	<0.001	Herd size
tested using gE)	November vs. March	47.82	39.84,55.81	<0.001	P < 0.001
	August vs. June	–8.01	–16.00,–0.03	0.049	
	November vs. June	25.52	17.54,33.52	<0.001	
	November vs. August	33.54	25.55,41.53	<0.001	
	>99cows vs. 31–65 cows	68.33	40.82,95.83	<0.001	
	>99cows vs. 66–99cows	52.08	26.57,77.61	<0.001	

[a] Co = Coefficient, i.e. the expected difference across the population.
* Denotes an interaction between two independent variables.

Table 5
Herds recording a change (negative to positive) in BVD bulk milk antibody status in August and November.

Herd identification	Calving season	Enterprise	BVD vaccination status (Date of vaccination)	Weanling status 180	March	June	August	November
					% inhibition			
39	Spring	Mixed	UV	Negative	27.93	43.45	58.37	60.42
54	Spring	Dairy	UV	Positive	43.65	25.32	54.69	60.96
67	Spring	Mixed	UV	Positive	19.59	49.72	72.74	62.59
92	Autumn	Mixed	UV	Negative	51.5	38.11	71.62	19.45
139	Spring	Dairy	V (April)	Negative	24.47	46.07	39.9	60.98
142	Spring	Mixed	V (September)	Positive	51.26	48.52	61.65	84.84
152	Spring	Mixed	UV	Negative	32.06	42.67	60.38	60.98
155	Autumn	Mixed	UV	Negative	35.48	47.11	58.6	48.28
172	Spring	Dairy	V (March)	Negative	39.58	43.92	46.71	57.75
184	Spring	Mixed	V January	Positive	35.68	43.3	58.11	30.01
263	Spring	Dairy	UV	Positive	51.35	54.17	81.58	83.54
273	Spring	Mixed	UV	Positive	20.5	36.05	76.7	85.83
275	Spring	Dairy	V (March)	Positive	54.24	35.95	71.09	74.98
278	Autumn	Dairy	V (January)	Positive	52.76	31.74	55.1	53.43
291	Spring	Dairy	UV	Negative	32.71	44.31	51.79	67.75

4. Discussion

The design of disease control strategies should be built upon local knowledge (Greiser-Wilke et al., 2003; Lindberg et al., 2006). This current study aimed to document levels of exposure to BVDV and BoHV-1 in the Irish dairy cattle population. Inclusion of both viruses allowed data on concurrent exposure to BVD and BoHV-1 to be investigated for the first time in Ireland. Although the current study has highlighted a high level of exposure to both BVDV and BoHV-1, levels are comparable to those reported for other regions (Garoussi et al., 2008; Guarino et al., 2008; Paton et al., 1998; Ståhl et al., 2002; Thobokwe et al., 2004; Van Wuijckhuise et al., 1998; Woodbine et al., 2009).

It has been reported that the level of antibodies to BVDV in milk is inversely related to the amount of milk produced (Niskanen et al., 1989). As the majority of herds included in the present study were spring-calving with peak lactation occurring approximately 9 weeks post-calving (Quinn et al., 2005), milk yield will reduce in the latter half of the year. An increase in bulk milk antibody readings might therefore be expected (Rikula et al., 2005), and indeed such a trend

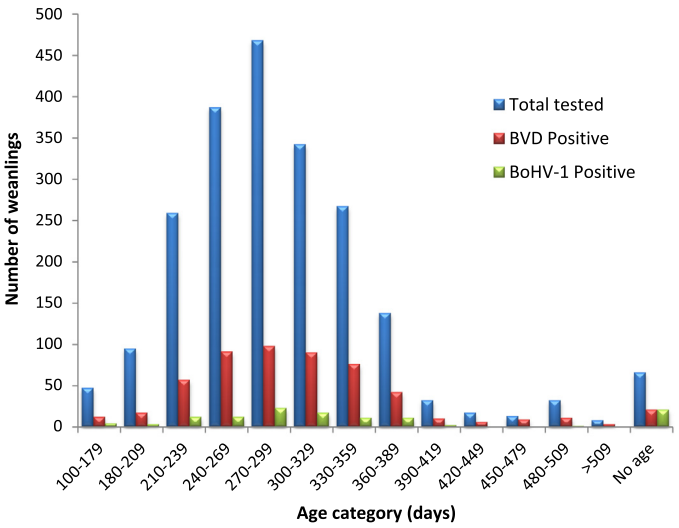

Fig. 3. Age and serological profile of study weanlings.

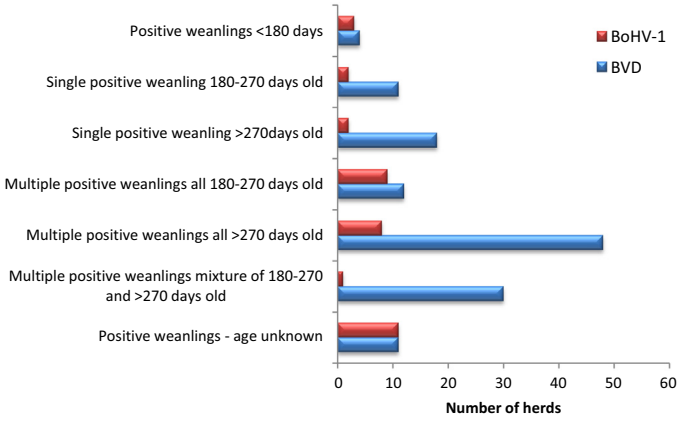

Fig. 4. Number of herds containing varying ages and levels of seropositive youngstock.

was highlighted in the current study, particularly in the case of BVDV. The increase, however, was not large, and in the majority of cases could not be considered biologically significant. Woodbine et al. (2009) reported similar results for BoHV-1 in England where a small but significant seasonal association was detected. It has been reported, however, that the total amount of secreted daily IgG is higher during peak milk production than later in lactation which may counteract any dilution effect of increased milk production, thereby contributing to the lack of major seasonal trends (Guidry et al., 1980; Nylin et al., 2000).

It should be noted, however, that in the case of four farms, more dramatic increases in BVD bulk milk readings were recorded between the June and August sampling. Housing is more often associated with spread of viral disease than is outdoor grazing (Ampe et al., 2012). An increased risk of BVDV infection over the summer months was unexpected, therefore, as Ireland is predominantly a pasture-based livestock system (Shalloo et al., 2004), the vast majority of cattle outdoors during the summer period. Outdoor grazing may, however, present a greater opportunity for trans-boundary transmission of BVDV between neighbouring farms. In addition, livestock movements have been associated with increased risk of disease spread (Gates et al., 2013), and as many landholdings in Ireland are highly fragmented (O'Donnell et al., 2008), livestock movements over the summer months will increase.

With regard to application of a suitable disease monitoring strategies in regions of high bulk milk seroprevalence for BVDV and BoHV-1, testing of more than a single annual sample in bulk milk seropositive herds would appear excessive in the short to medium term. More frequent bulk milk surveillance would, however, be of benefit in BVDV antibody negative herds, and possibly those herds in the lowest positive quartile (Fig. 4; 55–78% inhibition), especially over the summer months, to allow rapid intervention in cases of viral introduction.

There is value in documenting concurrent exposure to both BVDV and BoHV-1, with studies such as that undertaken by Risalde et al. (2013) outlining an increased severity in BoHV-1-associated tissue lesions in the presence of sub-clinical BVDV infection. The present study has highlighted, however, that although generation of concurrent disease status data is useful, application of a single surveillance strategy for both is not appropriate. The results presented here highlighted an expected association between BVD bulk milk antibody status and youngstock seropositivity based on previous

Table 6
Logistic regression analysis of BVD and IBR recent infection status (180 days and 270 days).

Dependent variable Independent variable	Odds ratio	Confidence interval (95%)	p value	Model (p value)	Model goodness of fit (p value)
BVD recent infection 180[a]					
66–99cows vs. 31–65 cows	1.80	0.93,3.47	0.079[d]	Herd size	p = 0.606
>99cows vs. 31–65cows	1.88	1.01,3.49	0.046	Calving season	
SA/YR vs. Spring	2.44	1.19,5.04	0.015	Annual BVD herd status	
BVD positive vs. BVD negative[c]	4.86	1.79,13.15	0.002	(p = 0.0002)	
BVD recent infection 270[b]					
Region 2 vs. region 1	0.56	0.32,0.98	0.041	Region	p = 0.970
SA/YR vs. Spring	2.39	1.15,4.96	0.019	Calving season	
BVD positive vs. BVD negative[c]	5.06	1.68,15.31	0.004	Annual BVD herd status (p = 0.0001)	
BoHV-1 recent circulation 180[a]					
66–99cows vs. 31–65 cows	7.11	0.86,58.57	0.068[d]	Herd size	p = 0.615
>99cows vs. 31–65cows	8.35	1.07,65.04	0.043	(p = 0.005)	
BoHV-1 recent circulation 270[b]					
Region 2 vs. region 1	0.40	0.15,1.06	0.066[d]	Region	p = 0.202
>99cows vs. 31–65 cows	7.52	0.91,62.03	0.061[d]	Herd size	
>99cows vs. 66–99cows	5.79	0.72,46.85	0.100[d]	(p = 0.034)	

[a] Recent infection herd classification based on the presence of at least one seropositive weanling over 180 days of age.
[b] Recent infection herd classification based on the presence of at least one seropositive weanling over 270 days of age.
[c] BVDV annual mean herd status.
[d] Included for the purposes of highlighting a trend.

Table 7
Logistic regression analysis of BVD and IBR vaccination status.

Dependent variable Independent variable	Odds ratio	Confidence interval (95%)	p value	Model (p value)	Model goodness of fit (p value)
BVDV vaccination					
66–99cows vs. 31–65 cows	2.08	1.11,3.89	0.023	Herd size	p = 0.299
>99cows vs. 31–65cows	2.61	1.40,4.88	0.003	Annual BVD herd status	
BVD positive vs. BVD negative[a]	4.12	1.87,9.07	<0.001	Annual IBR herds status	
BoHV-1 positive vs. IBR negative[b]	1.79	0.96,3.32	0.065[c]	IBR vaccination	
BoHV-1 vaccinated vs. unvaccinated	2.91	1.06,8.00	0.039	(p < 0.0001)	
BoHV-1 vaccination					
66–99cows vs. 31–65 cows	7.74	0.96,62.55	0.055[c]	Herd size	p = 0.494
>99cows vs. 31–65cows	15.11	1.98,115.36	0.009	Calving season	
SA/YR vs. Spring	2.40	0.94,6.12	0.067[c]	BVD vaccination	
BVD vaccinated vs. unvaccinated	3.63	1.32,9.93	0.012	(p < 0.0001)	

[a] BVD annual mean herd status.
[b] BoHV-1 annual mean herd status.
[c] Included for the purposes of highlighting a trend.

investigations of spot testing (Houe et al., 2006). The use of BVD spot test samples as a convenience sample for detecting the presence of BoHV-1 carriers in a herd, however, is not useful, with no association between BoHV-1 seropositive weanlings and BoHV-1 bulk milk antibody status having been highlighted. The prevalence of BoHV-1 seropositive youngstock in both dairy and beef herds tends to be low (Guarino et al., 2008; Romero-Salas et al., 2013; Waldner and Kennedy, 2008), thereby reducing the usefulness of this management group as herd sentinels. For the dairy herd therefore, where marker vaccines are used, bulk milk antibody status alone acts as a sufficient surveillance tool.

Given the similar methods of transmission of BVDV and BoHV-1, the occurrence of regional differences in a small country such as Ireland (agricultural land base is five million hectares approximately), having a large cattle population of approximately six million bovines (CSO, 2007) and poor farm-level biosecurity (Sayers et al., 2013), is noteworthy. Historically, BoHV-1 infections were very much associated with respiratory disease in beef units as opposed to dairy enterprises. Region-1, although the least cattle dense region of Ireland with regard to dairy livestock, has a much higher proportion of beef cattle, and farmers in this region are less likely to implement quarantine for purchased stock (Sayers et al., 2013). Both factors may contribute to the higher probability of being BoHV-1 bulk milk positive in this region. A similar conclusion has been suggested by Dias et al. (2013) where a predominantly dairying region of Brazil had the lowest apparent prevalence of BoHV-1 than other regions examined. This differs from BVDV, where it is accepted that the prevalence of BVDV is influenced by the regional cattle density (Almeida et al., 2013; Garoussi et al., 2008; Handel et al., 2011; Houe et al., 1995), and increased prevalence of BVDV is associated with higher cattle densities, supporting the findings of this current study.

Regional effects were also noted in RVC herds in models including youngstock of ≥270 days of age, with Region-2 less likely to have a seropositive weanling than Region-1. Although logical for BoHV-1 based on bulk milk findings, the increased likelihood of Region-1 herds having RVC for BVDV seems counterintuitive, and Region-2 having higher BVD bulk milk antibody readings. As foetuses of seropositive dams are rarely infected with BVDV, however (Brownlie et al., 1998), higher bulk readings should indicate greater foetal protection in these herds, leading to fewer PIs born and less youngstock exposed to BVDV. These findings are supported by a recent examination of the herd-level risk factors associated with the presence of BVDV on Irish farms, with herds in Munster (Region-2) being significantly less likely to produce one or more virus positive calves than Connaught (Region-1) (Graham et al., 2013). The model which included youngstock ≥180 days old did not yield a regional effect and may reflect interference from BVDV MDAs which have a relatively long half-life (Fulton et al., 2004).

In agreement with a number of other national and international studies (Boelaert et al., 2005; Cowley et al., 2011; Raaperi et al., 2010; Solis-Calderon et al., 2003; Van Wuijckhuise et al., 1998; Woodbine et al., 2009), a significant association between BoHV-1 infection/exposure and herd size was documented in this study, though not all countries report such a finding (Billinis et al., 2005). Larger herds also recorded higher ELISA readings in the current study. This may be due to increased within-herd prevalence in these herds as Raaperi et al. (2010) reported higher numbers of seropositive animals in larger herds. Additionally, larger herds have more susceptible animals available to maintain infection and herd size is a cluster variable for several biosecurity risks such as increased purchase of animals and increased visitors (veterinary practitioners, technicians, contract workers), all of which will increase the risk of disease introduction and maintenance (Boelaert et al., 2005; van Schaik et al., 1998). It is interesting to note that Sayers et al. (2013) reported that larger herds in Ireland are more likely to join a herd health scheme, which may reflect an awareness of the increased disease risk on these farms. Additionally, larger herds in the current study were also more likely to vaccinate for BVDV and BoHV-1, again highlighting an awareness of the increased disease risk in these herds.

The use of spot testing of youngstock is a well-established method of documenting recent BVDV circulation in a herd (Houe et al., 2006) to overcome the drawbacks of bulk milk analysis. To increase the usefulness of bulk milk analysis alone in highlighting recent BVDV circulation, Thobokwe et al. (2004) suggested a revised BVD ELISA positive cut-off of 80% inhibition using the Pourquier ELISA, a cut-off applied in a subsequent economic analysis (Heuer et al., 2007). In order to examine the applicability of such a strategy in another jurisdiction, a Wilcoxon rank sum analysis was applied in the current study to highlight differences in bulk milk analysis between herds recording positive and negative spot tests. While the findings in part aligned with those of Thobokwe et al. (2004), in that use of an elevated positive cut-off of 80% would be applicable to BVDV unvaccinated herds at certain times of the year, its use for vaccinated herds in Ireland is not appropriate. Interpretation of spot testing was stricter in this current study, however, which may account for the difference between both studies.

5. Conclusion

Prevalence studies have an important role to play in highlighting the necessity, or otherwise, for disease control and eradication schemes. This study highlighted high levels of exposure to BVDV and BoHV-1 in Irish dairy herds and also the lack of dramatic seasonal differences in bulk milk ELISA results. In the short to medium term, analysis of a single annual bulk milk sample would appear a

suitable surveillance strategy for both BVDV and BoHV-1 in Ireland, with spot testing required to highlight recent viral circulation in BVDV vaccinated herds.

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Appendix: Supplementary material

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